

THE EFFECT OF pH ON OXYGEN CONSUMPTION AND ACTIVITY IN THE BATHYPELAGIC MYSID *GNATHOPHAUSIA INGENS*

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The physical and chemical properties of the ocean at a given depth are relatively stable; however, there are appreciable depth-related gradients of many parameters. Among these are temperature, pressure, and oxygen concentration. The effects of these gradients on the physiology of midwater species has only recently begun to be investigated (Teal and Carey, 1967; Teal, 1971; Smith and Teal, 1973, Childress, 1969, 1971, 1975; Quetin and Childress, 1976). Among the most dramatic are the changes in oxygen concentration associated with the oxygen minimum zones that exist at intermediate depths in most of the world's oceans (Schmidt, 1925; Sewell and Fage, 1948; Banse, 1964). Gradients of pH values are associated with oxygen minima, and pH values may range from 8.3 at the ocean's surface to 7.5 or less in the oxygen minimum (more than a six-fold increase in acidity; Park, 1968). Although the importance of blood pH is well known, there is virtually no information available on the metabolic effects of water pH. The crustacean, *Gnathophausia ingens*, which resides in the oxygen minimum layer, was chosen for the study of this problem. This shrimp is a lophogastrid mysid whose respiratory and circulatory adaptations to low oxygen have been extensively studied (Childress, 1968, 1969, 1971, 1975; Belman and Childress, 1976). This report examines the effect of pH on oxygen consumption, oxygen removal from the respiratory stream, and activity in *G. ingens*.

MATERIALS AND METHODS

Specimens of *Gnathophausia ingens* were collected from basins off the coast of Southern California at depths of 400 to 900 meters with a midwater trawl. The animals were transported to the laboratory in aerated containers maintained at approximately 5° C. The mysid *G. ingens* was chosen as an experimental animal, because it can be maintained in the laboratory for long periods of time (Childress, 1971). All of the experimental animals were sexually immature, of undetermined sex, and had a wet weight between 3 and 13 g.

Oxygen consumption

Oxygen consumption rates for the mysid *Gnathophausia ingens* were measured in much the same way as Childress (1971). Animals were placed in a water-jacketed respirometer maintained at 5.5° C and covered to prevent the entrance of light. The rate of change of the partial pressure of oxygen in the respirometer

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was continuously monitored with a Clark-type oxygen electrode (Clark, 1956). The electrode was calibrated in air-saturated and nitrogen-saturated sea water (5.5° C) before and after each experiment. Any experiment in which the nitrogen calibration changed noticeably or the air calibration changed by more than 2% was not used.

In determining the effect of pH on respiration, two experimental procedures were followed. The first tested the effect of pH on oxygen consumption at low oxygen partial pressures. It was also used to estimate the limitation of activity by oxygen availability. This procedure required that an animal placed in the respirometer at a specified pH be allowed to consume all the oxygen present. The experiments that followed this procedure lasted approximately ten hours, depending on the size of the animal. Oxygen electrodes used in these experiments were calibrated in sea water of the same pH as the experiment. The second procedure was designed to show any short-term effect of pH on activity or rate of oxygen consumption without the stress of low oxygen. This procedure involved changing the water in the respirometer at five hour intervals and alternating sea water of pH 7.9 with that of either pH 7.1 or 8.7. These pH values were chosen because a tolerance over this range would also indicate a tolerance to fluctuations in pH which might occur in the environment. A total of five water changes was usually made during a single run. The oxygen electrodes used in these experiments were calibrated at pH 7.9 and the effect of pH on the calibration later determined. The difference in the calibrations was always less than 0.5% and was not subtracted in calculations of the respiratory rate.

In order to maintain constant pH throughout an experiment, it was necessary to buffer the sea water. Tris(hydroxymethyl)aminomethane (final concentration 20 mg/liter) adjusted to the required pH with either HCl or NaOH and diluted with distilled water so as to be isosmotic with salt water was found to be sufficient. To determine the effect of buffering the sea water, experiments of approximately four hours duration were done in both buffered and unbuffered sea water. During this period, the pH of the unbuffered water did not change greatly. Oxygen consumption rates in the buffered and unbuffered water were not significantly ($P \geq 0.1$, $n = 8$) different.

Bacterial growth was minimized by the addition of streptomycin sulfate (20 mg/liter) to the sea water. The remaining bacterial oxygen consumption was estimated by measuring the rate of oxygen consumption in the respirometer for 6–12 hours after the animal was removed. These rates were constant and always less than 5% of the total measured rate. The bacterial rates were subtracted in calculating the oxygen uptake rates of the animals.

Oxygen extracting ability

The ability of *G. ingens* to extract oxygen from sea water at different partial pressures was calculated from measurements of the oxygen content of sea water before and after passing through the gills. To measure oxygen in the exhaled water, an animal's head was placed in a plastic vial while a collar, cut from a rubber balloon, sealed the animal to the vial. The collar was loosely placed so as to not compress the carapace, and the flow of water through the vial was checked with a nontoxic dye. A microcathode (0.0152 mm diameter platinum cathode) oxygen

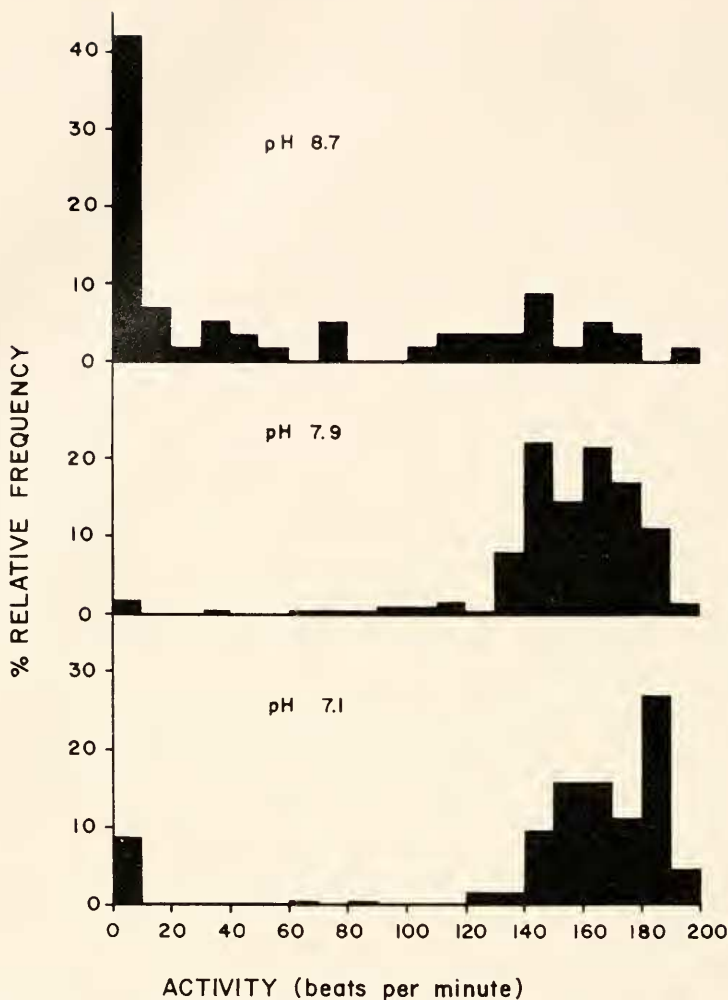


FIGURE 1. The relative frequencies of constant activities lasting at least ten minutes at pH 7.1, 7.9, and 8.7. There were 66 observations at pH 7.1, 206 at pH 7.9, and 57 at pH 8.7.

electrode, chosen because it is insensitive to stirring, was placed inside the vial, while another electrode in the bath recorded the oxygen content of inhaled water. A stirrer was placed in the bath to both stir the electrode and to keep the water evenly mixed.

Constant pH was maintained during this experiment by adding Tris(hydroxymethyl)aminomethane (10 mg/liter) to the water before the experiment. An experiment consisted of placing an animal in water of a specified pH and reducing the partial pressure of oxygen to approximately 6–10 mm Hg by bubbling nitrogen through the water. Constant stirring of the bath caused the partial pressure of oxygen to slowly return to approximately 100 mm Hg over a period of five hours. The pH of the water was then changed by slowly adding small aliquots of either

HCl or NaOH. Each animal was subjected to water of three different pH values, the order being changed for different animals to avoid "placement errors". The oxygen electrodes could be removed from the apparatus without disturbing the animal and were calibrated with each change of pH.

Activity

Activity was continuously recorded in all experiments. Individuals of *G. ingens* were held by the carapace in plexiglass "racks" (Quetin, Mickel, and Childress, 1978). A light-emitting diode light source and miniature photoresistor, both cast in clear epoxy, were placed opposite one another across the pleopods of an animal. The photoresistor functioned as one arm of a Wheatstone bridge. Each pleopod beat interrupted the light beam unbalancing the Wheatstone bridge, and thus generated an electrical pulse. The pulses were time-averaged by a cardiometer and recorded on a potentiometric chart recorder.

RESULTS

Activity and pH

The activity of the mysid *Gnathophausia ingens* was affected by pH. The distributions of constant activities, lasting at least ten minutes, from 40 runs on eight individuals is presented in Figure 1. As shown, the activity of animals in water of pH 7.1 and 7.9 was remarkably constant. At these pH values, individuals either swam at 140 to 190 beats per minute or did not swim. At pH 8.7 individuals more frequently did not swim and were generally less active than at the other pH values.

Individuals placed in water of pH 8.7 were observed to perform extensive cleaning behavior. This behavior consisted of repeated wiping of the antennae and mouthparts with the first several pairs of pereopods. During cleaning activity, the animal usually decreased its swimming activity and this most likely accounts for the trend toward lower and more variable activity at the higher pH.

Pleopod beat was not affected by oxygen concentration, and most animals continued swimming at relatively high rates for 15-30 minutes after the oxygen concentration in the respirometer became unmeasurably low.

Oxygen consumption and activity

Individuals of *G. ingens* were found to be capable of a wide range of oxygen consumption rates. Activity of individual *G. ingens* had a profound effect on oxygen consumption rate (Fig. 2). During a single run, rates could vary as much as ten-fold, from approximately $10 \mu\text{l O}_2/(\text{g wet weight}\cdot\text{hr})$ to $100 \mu\text{l O}_2/(\text{g wet weight}\cdot\text{hr})$. Most of this variation could be attributed to "spontaneous" changes in the animals' activity. Changes in activity during an experiment could completely mask any less subtle responses to the other parameters being tested. For this reason activity was recorded in all experiments.

Measurements of changes in oxygen consumption for short term changes in activity were difficult to make due to the lag in oxygen consumption with an increase in activity. Oxygen consumption rates were, therefore, calculated for periods

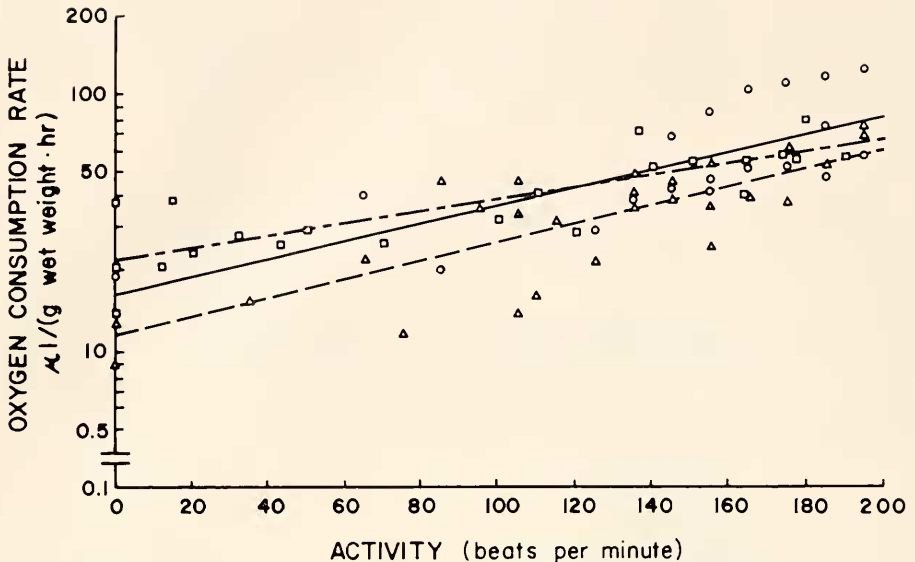


FIGURE 2. The relationship between activity (x , pleopod beats/minute) and oxygen consumption rate [y , $\mu\text{l O}_2/(\text{g wet weight} \cdot \text{hr})$] in *Gnathophausia ingens*. The regression line for pH 7.1 is $\log y = 1.2244 + 0.0035x$, has an r of 0.737 and is represented by the solid line. The data points at pH 7.1 are represented by circles. The regression line for pH 7.9 is $\log y = 1.0884 + 0.0036x$, has an r value of 0.851 and is represented by the uniformly dashed line. The data points at pH 7.9 are represented by triangles. The regression line for pH 8.7 is $\log y = 1.3571 + 0.0024x$, has an r value of 0.836 and is represented by the line of long and short dashes. The data points at pH 8.7 are represented by squares.

of constant activity, sustained for at least ten minutes and at partial pressures of oxygen above 20 mm Hg. By measuring rates at oxygen partial pressures above 20 mm Hg, it was assured that the animals were above their critical partial pressure of oxygen (Childress, 1971).

The relationship of respiratory rate to activity is presented in Figure 2. As shown, the relationship was found to be semi-logarithmic rather than linear. This indicates that the amount of oxygen consumed per pleopod beat increases with the rate of pleopod beat. The mean respiratory rate of nonswimming animals, including all pH values, was $19.3 \mu\text{l O}_2/(\text{g wet weight} \cdot \text{hr})$, $n = 32$, $s.d. = 14.5$. The rates for animals swimming at 140 to 190 pleopod beats per minute, taken from Figure 2, are from 39.1 to $77.5 \mu\text{l O}_2/(\text{g wet weight} \cdot \text{hr})$.

Oxygen consumption and pH

The effect of pH on respiration in *G. ingens* was studied in eight individuals from 3.9 to 9.3 g wet weight, in a series of 40 runs. The large variation in respiratory rate due to changes in activity made it difficult to choose a representative value for the respiratory rate of an animal during an experiment. For this reason, the relationship between activity and respiration for all animals was compared at three pH values (Fig. 2). Respiratory rates for each animal were grouped according to

activity (*c.g.*, all rates at activity 100 to 110 pleopod beats per minute) and the means taken. Each point on Figure 2 is the mean rate for one animal at the mean activity and pH.

Analysis of the regression line of respiration on activity for the three pH values showed no significant ($P > 0.1$, F-test) difference between the slopes of the lines. A test for homogeneity of variance, however, showed that the variance at pH 8.7 was significantly larger ($P < 0.05$, Bartlett's test) from that at pH 7.9 and pH 7.1. Due to this difference in variances the data could not be pooled, and therefore a regression line for each pH is shown (Fig. 2). The slopes of all the regression lines are significantly ($P < 0.001$, *t*-test) different from zero.

The effect of pH on oxygen uptake at oxygen partial pressures of 10–30 mm Hg was studied in five animals. The mean respiratory rates and standard deviation in water of pH 7.1, 7.9, and 8.7 are, respectively: 34.33 ± 15.5 , 30.07 ± 20.12 , $27.16 \pm 16.08 \mu\text{l O}_2/(\text{g wet weight}\cdot\text{hr})$. No significant ($P > 0.1$, *t*-test) difference between these means was found.

The critical partial pressure of oxygen (P_c) for *G. ingens* was found to be unaffected by pH (Fig. 3). The P_c values for 11 individuals at three pH values all fell within the 95% confidence interval for the relationship between regulated oxygen consumption rate and P_c found for *G. ingens* (Childress, 1971).

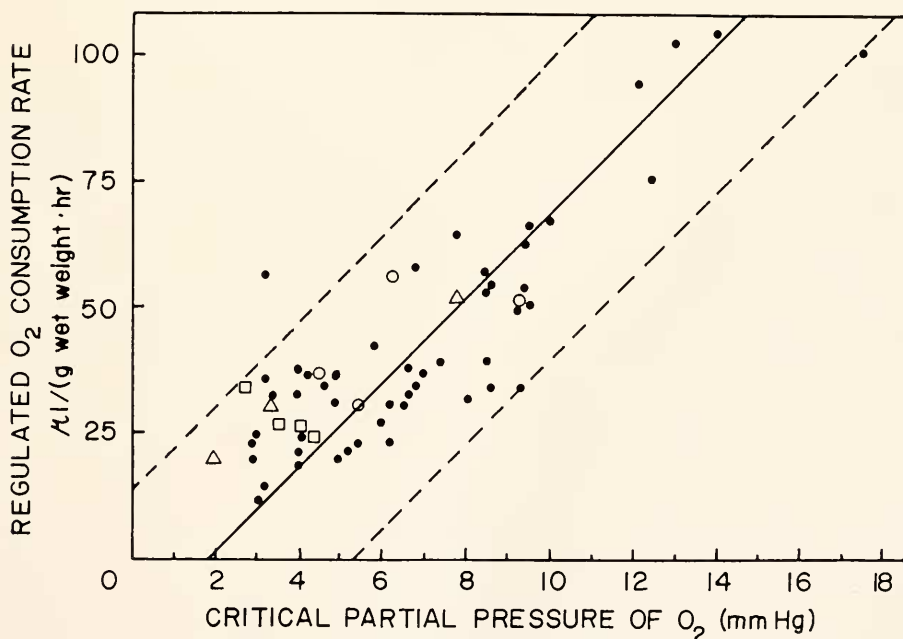


FIGURE 3. The relationship between oxygen consumption and P_c in *Gnathophausia ingens*. The regression line ($x = 4.973y + 1.798$, solid line), 95% confidence intervals for an individual x at a given y (dashed lines) and the solid circles which these relationships describe are taken from Childress (1971). These observations were made at pH values of approximately 8 to 8.3. The data points from the present study are represented by triangles for pH 7.1, circles for pH 7.9 and squares for pH 8.7.

TABLE I

The ability of *G. ingens* to extract oxygen from water, at three pH values, expressed as the percentage of oxygen content of inhaled water. Values are means for percentage of oxygen extracted at oxygen partial pressures of 10–40 mm Hg and 40–80 mm Hg. The numbers in parentheses are the number of observations followed by standard error of the mean.

pO ₂ (mm Hg)	pH of observations					
	7.1		7.9		8.7	
	10–40	40–80	10–40	40–80	10–40	40–80
Animal						
1	62.8 (6, 3.6)	50.6 (9, 0.7)	51.7 (11, 3.9)	38.3 (10, 1.9)	58.4 (4, 2.6)	46.3 (13, 3.1)
2	70.5 (5, 3.6)	32.5 (5, 2.1)	58.7 (8, 5.6)	25.4 (10, 2.6)	58.5 (7, 3.6)	26.6 (18, 2.1)
3	52.7 (5, 9.4)	54.8 (12, 1.4)	37.2 (13, 3.2)	46.0 (19, 0.8)	30.4 (4, 4.2)	48.7 (21, 2.1)
4	51.6 (9, 6.2)	42.3 (13, 1.2)	39.8 (12, 3.5)	37.8 (19, 1.5)	21.9 (7, 2.6)	36.7 (18, 2.3)
5	72.5 (19, 1.2)	63.2 (17, 2.2)	45.7 (12, 2.3)	43.8 (22, 1.8)	6.0 (13, 3.2)	52.6 (21, 1.0)
6	71.9 (22, 1.4)	67.4 (10, 1.8)	53.9 (5, 4.6)	51.3 (14, 3.0)	64.3 (19, 1.8)	49.7 (19, 2.3)

Oxygen extracting ability and pH

The ability of individual *G. ingens* to extract oxygen from water was measured in 18 runs with six individuals ranging in size from 5–8 g wet weight. The results for the six animals are summarized in Table I. The values in Table I are means of the values for the percentage of O₂ extraction in the ranges of oxygen partial pressures of 10–40 mm Hg and 40–80 mm Hg. Standard errors given in the table are not meaningful because, as can be seen in Figure 4, the percentage extraction declines continuously at higher oxygen partial pressures. Therefore, standard errors would indicate the range of values for the percentage utilization over the oxygen concentrations tested, rather than the variability of sampled values. This would overestimate the variability found. While individual shrimp differed in the absolute values for the percentage oxygen extraction, general trends for responses to both changes in pH and oxygen concentration could be found. The results from a representative experiment are shown in Figure 4.

The typical response was moderate extraction (25–55%) at partial pressures of oxygen above 40 mm Hg. As the partial pressure of oxygen further declined, the percentage extraction increased to 75–85% and in some cases reached a peak at oxygen partial pressures of 10–15 mm Hg and then declined with a further decrease in oxygen concentration. This result is similar to that found by Childress (1971).

No statistical difference could be found between the values for the percentage O₂ extraction at pH 7.9 and 8.7 ($P > 0.1$), but each of the *G. ingens* that was studied extracted a significantly ($P < 0.05$, Mann-Whitney test) greater percentage of oxygen in water of pH 7.1 than in water of pH 7.9 or pH 8.7. As Figure 4 shows, increased O₂ extraction at pH 7.1 occurred over the whole range of oxygen partial pressures and apparently was not related to the stress of low oxygen.

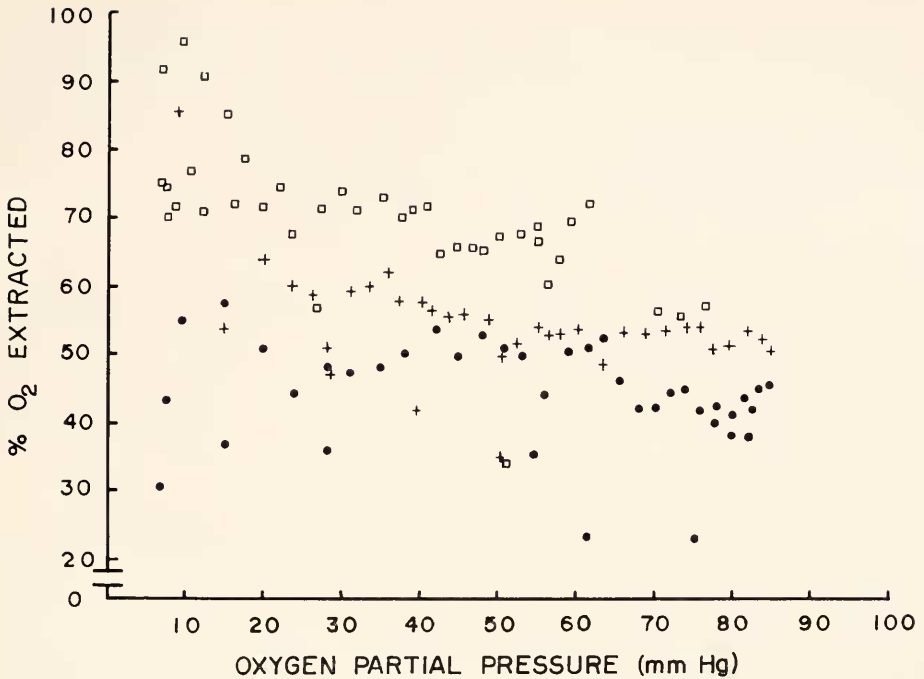


FIGURE 4. The oxygen extracting ability of a single *G. ingens*, at three pH values, expressed as the percentage of oxygen in inhaled water: squares, pH 7.1; solid circles, pH 7.9; and crosses, pH 8.7.

DISCUSSION

The relationship between activity and metabolism has been only slightly investigated in crustaceans. In one study of the mysid, *Mysis relicta*, it has been concluded that activity during vertical migration has no effect on metabolism (Foulds and Roff, 1975). Another study concludes that oxygen consumption of euphausiids remains the same regardless of whether the animal is swimming or not (Lasker, 1966). On the other hand, Halcrow and Boyd (1967) found a linear relationship between oxygen consumption and swimming activity in the amphipod *Gammarus oceanicus*, and Ivlev (1963) found a semi-logarithmic relationship between oxygen consumption and swimming velocity in the shrimp *Leander adspersus*. The data collected in this study show a quite significant relationship between activity and metabolic rate in *Gnathophausia ingens*. The exponential relationship between rate of pleopod beat and oxygen consumption in this species is comparable to that found for the relationship between rate of cirral beat and oxygen consumption in the barnacle *Balanus balanoides* (Newell and Northcroft, 1965). Clarification of the nature of the relationship between swimming velocity and oxygen consumption in *G. ingens* awaits the determination of the propulsive efficiency of the pleopod beat. It is clear from this study however, that the rate of aerobic metabolism in this species is quite closely related to its activity level. Further, activity certainly constitutes a major fraction of the overall energy usage of this entirely pelagic species.

At the relatively high activities exhibited by individuals during experiments, respiratory rates could range from 30 to 80 $\mu\text{l O}_2/(\text{g wet weight}\cdot\text{hr})$. Whether these rates of activity can be maintained in the oxygen minimum layer can be calculated by assuming an oxygen concentration of 0.25 ml/liter and a ventilation flow rate of 240 ml/(g wet weight \cdot hr) (Childress, 1971). With O_2 extraction of 85%, individuals of *G. ingens* can extract enough oxygen to sustain rates of activity of 138 and 172 pleopod beats per minute at pH 7.1 and pH 7.9.

The most striking aspect of the data on this species is that there are no dramatic effects of pH on its respiratory processes. That is, this shrimp seems to regulate its metabolism over a wide range of pH values. For example, the relationship between activity and oxygen consumption is unaffected by pH. Further, pH appears to have little effect on activity at the two lower and environmentally more realistic values. Level of pH also apparently does not alter the ability of this species to regulate its oxygen consumption at the low environmental oxygen concentrations where it normally lives. This is shown by the fact that the relationship between critical partial pressure and regulated oxygen consumption rate found by Childress (1971) is unaffected by pH over the range tested.

The one case where pH had a strong effect involved the extraction of oxygen from the respiratory stream. The data show quite clearly that the percentage of O_2 extraction is 10% to 30% higher at pH 7.1 as compared to 7.9 and 8.7. Since the utilization at pH 7.1 is elevated at both high and low oxygen partial pressures, this is apparently not the result of a stress on the oxygen uptake systems of the animal forcing it to increase its extraction to maintain a constant oxygen consumption. Paradoxically this higher percentage of extraction at low pH apparently does not improve the ability of *G. ingens* to regulate its oxygen uptake (Fig. 4). This implies that there is a loss in effectiveness of uptake at some other site as a result of the lower pH. The question of how the percentage of extraction of this species can be higher at limiting oxygen partial pressures at pH 7.1 as compared to pH 7.9 and pH 8.7, however, is still left unanswered. Further studies on the tolerance of vertically-migrating animals to low pH may be interesting because pH may be as important a factor as oxygen in limiting the distribution of some species in relation to low oxygen regions.

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SUMMARY

1. Pleopod beat of *G. ingens* was unaffected by pH at pH 7.1 and pH 7.9 but was lower at pH 8.7 due to increased cleaning activity.
2. The relationship between oxygen consumption rate, and pleopod beat was found to be semi-logarithmic.
3. The relationship between oxygen consumption rate and activity was unaffected by pH in the range of pH 7.1 and pH 8.7.

4. The per cent O₂ extraction of oxygen by *G. ingens* was not statistically different at pH 7.9 and pH 8.7, but was greater at pH 7.1.
5. The ability of *G. ingens* to regulate its oxygen consumption was unaffected by pH in the range studied.
6. Since the increase in per cent O₂ extraction at pH 7.1 does not improve the ability of *G. ingens* to regulate its oxygen uptake, it appears that there is a loss in effectiveness elsewhere in its respiratory system at this pH.

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